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ANTIBIOTIC-ENHANCED PHAGOCYTOSIS OF 'BORRELIA RECURRENTIS' BY B--ETC(U)
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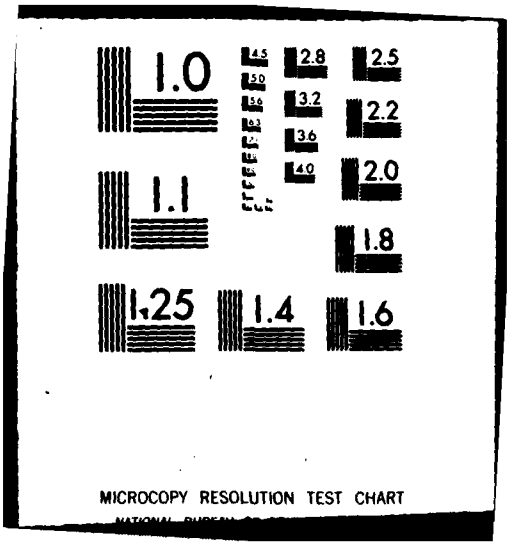
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(6) Antibiotic-enhanced Phagocytosis of Borrelia recurrentis by Blood Polymorphonuclear Leukocytes.

by

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Abstract. The removal of Borrelia spirochetes from the blood in relapsing fever was studied by examining patients' blood phagocytic cells with the Dieterle silver stain. Polymorphonuclear leukocytes ingested Borreliae at increased rates for several hours after antibiotic treatment, during which time the total numbers of circulating plasma spirochetes were decreasing. Incubation of infected blood at 37°C for 2 hours resulted in a progressive increase in phagocytosis. Addition of penicillin G and tetracycline to infected blood caused a further enhancement of phagocytosis. Electron microscopy of polymorphonuclear leukocytes revealed spirochetes in phagosomes. This antibiotic-enhanced phagocytosis of Borreliae by blood polymorphonuclear leukocytes has not been described in other bacterial infections and may explain, in part, the mechanism of the Jarisch-Herxheimer-like reaction after treatment of relapsing fever.

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Introduction

Louse-borne relapsing fever is an epidemic febrile and sometimes fatal illness, caused by the spirochete Borrelia recurrentis. The spirochetes circulate in plasma in high densities of about 10,000 - 100,000 per mm^3, allowing the diagnosis of this disease by microscopic examination of peripheral blood smears stained with aniline dyes. Antibiotic treatment is effective in clearing the blood of spirochetes within several hours. It also provokes a Jarisch-Herxheimer-like reaction, characterized by a rigor, a rise in temperature, and a drop in blood pressure (1). The fate of spirochetes after antibiotic treatment is unknown. Phagocytosis of organisms by blood polymorphonuclear leukocytes was suggested by Schofield et al (2) because of the leukopenia and cell vacuolation observed in patients after therapy, but blood smears stained with aniline dyes have not consistently revealed intracellular spirochetes. The observation by Faine et al (3) that phagocytosed leptospires were demonstrable intracellularly with a silver stain led us to apply the Dieterle silver stain (4) to blood smears of patients with relapsing fever. Organisms were frequently seen in polymorphonuclear leukocytes and the numbers of cells containing the organisms increased after antibiotic treatment.

Methods

Patients. Eleven Ethiopian adult males with fever and blood smears showing spirochetes in the plasma with Wright's stain were selected. None had received antibiotics before the study. Single dose antibiotic regimens were administered: erythromycin stearate 500 mg orally to 3 patients, tetracycline hydrochloride 500 mg orally to one and 250 mg intravenously to 3 patients, and procaine penicillin G 600,000 units intramuscularly to 4 patients.

Blood smears. Repeated blood smears were obtained hourly until negative for spirochetes. Blood smears, which had been taken before treatment, during treatment nearest to the time of onset of rigors, and at the time plasma spirochetes stainable with Wright's stain had been cleared, were selected for restaining by Dieterle's method (4). The rate of phagocytosis was measured by recording the number of polymorphonuclear leukocytes containing spirochetes per 100 cells counted.

In vitro phagocytosis. Blood from two patients with blood smears positive for spirochetes was drawn into heparin (final concentration 75 units per ml) and cooled to 4°C. Several hours later the blood was pipetted into sterile plastic tubes containing potassium penicillin G (final concentration 100 units/ml) or tetracycline hydrochloride (final concentration 100 μg/ml) or no antibiotic. The tubes were incubated at 37°C and inverted every 15 minutes. Specimens were removed at intervals of 15, 30, 60, and 120 minutes after the start of incubation for counting spirochetes.

Electron microscopy. After 120 minutes, incubation was stopped and a leukocyte-rich fraction was separated by centrifuging the blood at 100 x g for 5 minutes and then centrifuging the supernatant at 500 x g for 15 minutes. Glutaraldehyde 2% in 0.05 M phosphate buffer at pH 7.4 was added to the pellet. After 48 hr, the glutaraldehyde was poured off and replaced by phosphate buffer. The specimens were stored at 4°C until prepared for electron microscopy.

Results

The Dieterle stain of blood smears gave good definition of both extracellular spirochetes and spirochetes that were within polymorphonuclear leukocytes

(Figure 1A). The organisms appeared intracellular by their planes of focus and were occasionally coiled or compacted within cytoplasmic vacuoles. Leukocytes containing spirochetes were seen more often in smears obtained after than before antibiotic treatment (Table 1). At a time when blood smears stained with Wright's stain were read as negative for plasma spirochetes, approximately one-quarter of polymorphonuclear leukocytes contained spirochetes, although these organisms had not been discernible within cells stained with Wright's stain.

In order to determine whether leukocytes phagocytose plasma spirochetes in vitro, we incubated heparinized infected blood at 37°C. We observed a progressive increase in the rates of phagocytosis during two hours of incubation, and this increase was accelerated by the addition of penicillin G and tetracycline (Table 2). At times of 60 and 120 min after incubation in Patient 1 and after 30 and 60 min in Patient 2, the rates of phagocytosis were increased significantly by the addition of antibiotic ($p<0.05$). Blood incubated with antibiotics at 4°C showed no increase in phagocytosis. Spirochetes that were not phagocytosed exhibited no signs of lysis or fragmentation in the presence of the antibiotics and examination of these organisms with phase contrast microscopy showed an active pattern of normal motility.

To ascertain the intracellular locations of spirochetes within polymorphonuclear leukocytes we examined leukocyte-rich fractions of blood by electron microscopy. When blood was examined both from patients after therapy and after incubation of blood with antibiotics, intact spirochetes had been phagocytosed and were located within membrane-bound phagosomes. Digestion of spirochetes within the vacuoles was observed after 2 hours of incubation in the presence of antibiotic (Figure 1B, C).

Discussion

These studies show that the rapid removal of large numbers of plasma spirochetes from the blood of patients with relapsing fever is accompanied by an increased rate of phagocytosis of the spirochetes by circulating polymorphonuclear leukocytes. Fixed phagocytic cells of the reticulo-endothelium system in the liver, spleen, and bone marrow may also participate in the removal of spirochetes, but this was not studied in these patients. The site of this accelerated phagocytosis is presumably intravascular and likely resembles the intravascular surface phagocytosis of other bacteria by granulocytes described by Wood et al (5). This mechanism of phagocytosis did not require specific antibody and consisted of adherence of granulocytes to vascular endothelium with entrapment of bacteria between granulocytes and in fibrin meshworks.

The acceleration of intravascular phagocytosis following antimicrobial treatment that we observed in this study has not been described in other bacterial infections. Antibiotics might simply kill the spirochetes, thus rendering them more susceptible to phagocytosis. This mechanism does not seem likely, however, because the enhanced phagocytosis was observed in less than an hour after addition of antibiotic. Besides, the spirochetes showed no apparent changes in morphology or loss in motility after exposure to antibiotic. Thus, the antibiotics could act by other mechanisms, such as by altering the surface of the spirochetes to make them more adherent to leukocytic membrane structures.

The enhancement of phagocytosis after antibiotic treatment in relapsing fever may aid in explaining the Jarisch-Herxheimer-like reaction. The onsets of the Jarisch-Herxheimer-like reaction and the increased rate of phagocytosis of spirochetes both occur within one to 4 hours after

institution of antibiotic treatment. Polymorphonuclear leukocytes are known to release endogenous pyrogen after phagocytosis of other bacteria (6), and endogenous pyrogen may be one of the mediators of the rigor and temperature rise in the Jarisch-Herxheimer reaction (2). Release of other biologically active molecules by phagocytic cells might also participate in the mediation of the intravascular coagulation and hypotension (7) that accompany the Jarisch-Herxheimer-like reaction in patients with relapsing fever.

Table 1. Rates of phagocytosis of Borrelia recurrentis by blood polymorphonuclear leukocytes in 11 infected patients as measured by the proportion of polymorphonuclear leukocytes in blood smears containing silver-stained spirochetes.

Time of Examination	Mean Hours After Antibiotic Treatment	Number of Polymorphonuclear Leukocytes (per 100 counted) Containing Spirochetes	
		Range	Mean
Before antibiotic treatment*	0	0-22	5.3
Jarisch-Herxheimer-like reaction**	2.1	8-52	22.7***
Clearance of Extracellular spirochetes in Wright's stained smears	5.8	0-55	24.6***

* Antibiotic treatment was erythromycin stearate 500 mg orally in 3 patients, tetracycline hydrochloride 250 mg intravenously in 3 patients, tetracycline hydorchloride 500 mg orally in 1 patient, and procaine penicillin G 600,000 units intramuscularly in 4 patients.

** A Jarisch-Herxheimer-like reaction, heralded by rigor and temperature rise, occurred in 9 patients.

*** Mean values were significantly greater than the mean number of polymorphonuclear leukocytes containing spirochetes before antibiotic treatment by Student's t test ($p<0.05$).

Table 2. Effect of *in vitro* incubation of heparinized whole blood from two patients for 2 hours at 37°C and the addition of antibiotics* to the blood on the rate of phagocytosis of *Borrelia recurrentis* by polymorphonuclear leukocytes.

Time After Start of Incubation in minutes		Number of Polymorphonuclear Leukocytes Containing Silver-stained Spirochetes**		
		No Antibiotic	Penicillin G	Tetracycline
Patient 1***	0	5.3±0.7	-	-
	15	3.7±0.9	5.3±0.9	4.3±1.5
	30	7.0±3.0	13.0±5.3	11.3±2.3
	60	11.3±0.9	19.0±1.5†	22.7±2.3†
	120	12.7±2.9	31.7±0.7†	26.7±4.1†
Patient 2***	0	8.7±0.7	-	-
	15	13.0±4.4	20.7±4.7	22.7±3.9
	30	31.0±4.9	35.3±2.9	46.7±3.9†
	60	27.3±1.7	32.7±3.3	40.0±2.3†
	120	34.0±4.9	43.7±3.2	39.0±1.2

* Antibiotics were added in 0.9% NaCl in a volume one-tenth that of the blood to give final concentrations of potassium penicillin G 100 units/ml and tetracycline hydrochloride 100 µg/ml.

** Values are the means and standard errors of three independent countings of 100 polymorphonuclear leukocytes.

*** The blood of patient 1 contained approximately 1 spirochete per polymorphonuclear leukocyte and the blood of patient 2 contained approximately 13 spirochetes per polymorphonuclear leukocyte.

† Values that were significantly greater by Student's t test ($p<0.05$) in the antibiotic-treated specimens than in the specimens without antibiotic examined at the same time.

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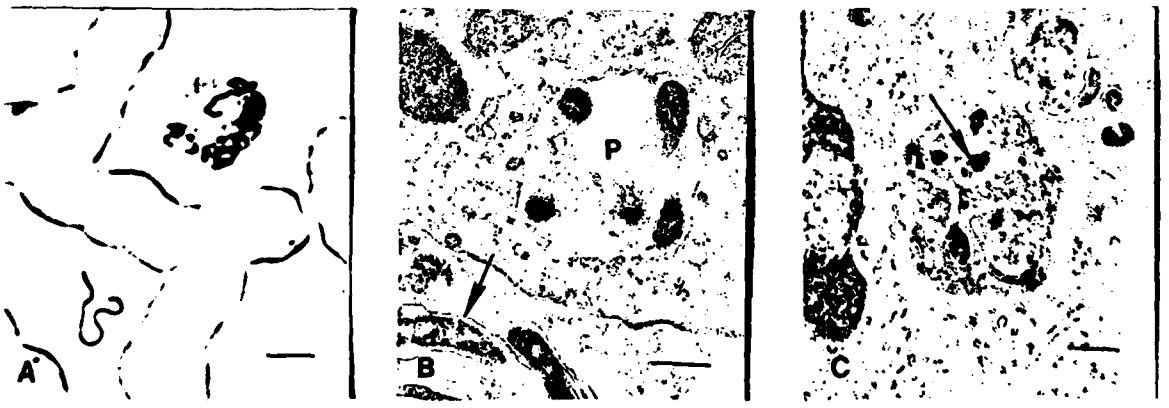
Figure Legend

Figure 1. Blood of patients with relapsing fever showing phagocytosis of spirochetes by polymorphonuclear leukocytes.

A. Blood smear of a 25 year old male patient who had been treated with erythromycin stearate 500 mg orally 6 hours previously. The smear, stained with Dieterle's silver stain, reveals extracellular spirochetes and spirochetes within a polymorphonuclear leukocyte, 42 per cent of which contained stainable spirochetal forms. A smear obtained one hour later was negative for extracellular spirochetes. Bar equals 5μm.

B. Leukocyte-rich fraction of blood from a patient with relapsing fever 2 hours after incubation with penicillin G 100 units/ml. Borreliae in cross-section are located both extracellularly (lower left) and intracellularly in a phagosome (P) of a polymorphonuclear leukocyte. Bar equals 0.5μm.

C. Phagolysosome contains digested remnants of a spirochete (arrow). Bar equals 0.5μm .



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